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ORIGINAL ARTICLE

Proprotein convertase subtilisin/kexin 9 (PCSK9) in patients with diffuse systemic sclerosis: A marker of disease activity and severe disease manifestations with potential therapeutic implementations

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ABSTRACT

Objectives: This study aims to investigate proprotein convertase subtilisin/kexin 9 (PCSK9) in patients with diffuse systemic sclerosis (d-SSc) and its relation to disease activity, severity and subclinical atherosclerosis in such group of patients.

Patients and methods: Between December 2019 and July 2021, a total of 41 patients with d-SSc (17 males, 24 females; mean age: 36.1±1.9 years; range, 19 to 58 years) and 41- age and sex-matched healthy controls (17 males, 24 females; mean age: 40.1±1.7 years; range, 20 to 60 years) were included. Disease activity and skin thickness of the patients were evaluated using the European Scleroderma Study Group (EScSG) score and modified Rodnan skin score (mRSS), respectively. Serum PCSK9 and carotid intima-media thickness (CIMT) were measured using enzyme-linked immunosorbent assay (ELISA) and Duplex ultrasound, respectively.

Results: Serum PCSK9 was higher in patients compared to controls (p=0.003), particularly in those with digital ulcer (DU) and interstitial lung disease (ILD) (p<0.001). The PCSK9 positively correlated with the mean pulmonary artery pressure, EScSG, mRSS, C-reactive protein (p<0.001), erythrocyte sedimentation rate (p<0.05), lipid profile, and mean CIMT (p<0.01). In the multivariate analysis, EScSG, mRSS, lipid profile, and waist circumference were significantly correlated with PCSK9. Serum PCSK9 levels of (182.6 ng/mL) had 77.7% sensitivity and 81.2% specificity for diagnosing DU versus (172.8 ng/mL) 90.1% and 73.5% for ILD (p<0.001).

Conclusion: Serum PCSK9 is upregulated in d-SSc with higher levels in severe disease manifestations such as DU and ILD. It is correlated well with disease activity, more severe disease manifestations, and CIMT. The PCSK9 inhibitors may be a target of therapy in diseases with premature atherosclerosis such as d-SSc regardless of its anti-cholesterol effect, at least in more severe manifestations.

Keywords: Atherosclerosis, diffuse systemic sclerosis, disease activity, PCSK9, proprotein convertase subtilisin/kexin 9.

Diffuse systemic sclerosis (d-SSc) is a rare connective tissue disease which significantly affects quality of life with high mortality rates.[1] There is increasing evidence of higher atherosclerosis in d-SSc patients compared to healthy individuals.[2] Suggested mechanisms of atherosclerosis include chronic inflammation, altered lipid profiles, autoantibodies, and endothelial dysfunction.[3]

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease mainly synthesized in the liver. It enhances cardiovascular (CV) risk by decreasing the clearance of low-density lipoprotein (LDL) and acting as a regulator of atherogenic inflammation.[4] Several studies have examined the relation between PCSK9 and various autoimmune disorders such as rheumatoid

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arthritis (RA),[5] systemic lupus erythematosus (SLE),[6] and spondyloarthropathies (SpA).[7] However, the information of its role in patients with d-SSc is still lacking.

In the present study, we aimed to investigate PCSK9 in patients with d-SSc and its relation to disease activity, severity and subclinical atherosclerosis in such group of patients.

PATIENTS AND METHODS

This case control study was conducted at Cairo University, Faculty of Medicine, Department of Internal Medicine, Rheumatology and Clinical Immunology Unit between December 2019 and July 2021. A total of 41 patients with d-SSc (17 males, 24 females; mean age: 36.1±1.9 years; range, 19 to 58 years) diagnosed according to the American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) criteria 2013[8] and 41- age and sex-matched healthy controls (17 males, 24 females; mean age: 40.1±1.7 years; range, 20 to 60 years) were included. All patients underwent a detailed clinical history and a complete physical examination including skin examination using the modified Rodnan skin score (mRSS).[9] Disease activity was assessed using the European Scleroderma Study Group (EScSG) score.[10] Patients with obesity, hypertension, diabetes mellitus, ischemic heart disease, peripheral vascular disease, stroke, recent infections and other autoimmune diseases such as RA, SLE, SpA, antiphospholipid syndrome, Sjögren syndrome and mixed connective tissue disease were excluded from the study.

For all participants, laboratory investigations including serum total cholesterol (TC), LDL-C, high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), fasting blood glucose (FBG), liver and kidney functions, complete blood count (CBC) and inflammatory reactants, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured using standard methods. Serum PCSK9 levels were measured by enzyme-linked immunosorbent assay (ELISA) (CUSABIO kit, Catalog Number: CSB-EL017647HU, Houston, USA).

Duplex ultrasound examination was performed to asses carotid intima-media thickness (CIMT) using a B-mode ultrasound (Acuzon Phillips, Germany) with a 7.5 MHz linear array imaging probe. All examinations were done by a single radiologist. The ultrasound images were obtained 1 cm proximal to carotid bifurcation. Upper normal average intima-media thickness (IMT) is estimated to be up to <0.8 mm with atherosclerotic plaques defined as a thickness greater than 1.5 mm as measured from the media-adventia interface to the intima-lumen interface.[11] Chest X-ray, high-resolution computed tomography (CT) chest and echocardiography were done to all patients for detection of interstitial lung disease (ILD) and pulmonary hypertension (PHT).

Statistical analysis

Statistical analysis was performed using the SPSS version 17.0 (SPSS Inc., Chicago, IL, USA), GraphPad Prism (version 7, California, USA) and Microsoft Excel, version 10 software. The Kolmogorov-Smirnov test was used to test normality. Data were expressed in mean ± standard error (SE), median (min-max) or number and frequency, where applicable. Comparison between the groups was performed by the unpaired Student t-test (quantitative data) and chi-square test (categorical data). The associations between variables were assessed using the Pearson correlation coefficient. Multiple regression analysis was performed to identify factors associated independently with PCSK9. Finally, receiver operating characteristic (ROC) curve was drawn to evaluate PCSK9 diagnostic value in diagnosing ILD and digital ulcer (DU) which are considered severe disease manifestations with high morbidity and mortality. Cut-off values were determined such that they maximized the sum of sensitivity and specificity. A p value of <0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics of the patients and controls are shown in Table 1. Compared to healthy controls, plasma levels of TC, TG, LDL-C levels and ESR were significantly higher, while HDL-C was significantly lower in d-SSc patients. The d-SSc patients had a higher mean serum PCSK9 levels (146.7±6.8 ng/mL)

compared to control group (115.3±7.4 ng/mL, p=0.003). The mean CIMT in d-SSc patients was (0.99±0.05 mm) versus (0.87±0.02 mm) in the control group (p=0.006). Serum PCSK9 in patients with DU (n=9) and ILD (n=11) were significantly higher compared to those without these manifestations (194.2±5.2) and (190.6±4.9) versus (133.4±7.1) and (130.6±7.2), respectively (p<0.001).

Serum PCSK9 levels correlated negatively with HDL-C (r=-0.35, p=0.02) and positively with waist circumference (WC) (r=0.5, p<0.001), mean pulmonary arterial pressure (r=0.3, p=0.045), LDL-C (r=0.65, p<0.001), TG (r=0.37, p=0.01), ESR (r=0.23, p<0.05), CRP (r=0.46, p<0.001), mRSS (r=0.9, p<0.001), EScSG score (r=0.87, p<0.001) and mean CIMT (r=0.5, p<0.001) (Table 2, Figures 1 and 2).

The ROC curve analysis showed that, for the diagnosis of DU, the value of area under the curve (AUC) for PCSK9 was 0.87 (95% confidence interval [CI]: 0.77-0.97; p=0.0007; sensitivity= 77.7%; specificity= 81.2%) with a cut-off value of 182.6 ng/mL. For diagnosing ILD, the value of

Table 1. Demographic, clinical, and laboratory data of participants

	d-SSc patients (n=41)			Controls (n=41)			
Parameters	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (year)			36.1±1.9			40.05±1.7	0.5
Weight (kg)			61.5±1.5			71.1±1.5	<0.001*
Height (cm)			161.8±1.1			166.4±1.7	0.03
Waist circumference (cm)			75.7±1.5			79.7±0.9	0.032*
Pulse (beats/min)			87.3±1.4			87.2±1.6	0.9
Systolic blood pressure (mmHg)			113.1±2.1			123.2±3.2	0.012*
Diastolic blood pressure (mmHg			71.2±1.3			76.6±1.8	0.022*
TC (Normal: <200 mg/dL)			204.9±11.9			161.1±9.6	0.006*
TG (Normal: 53-150 mg/dL)			132.6±5.9			113.7±5.3	0.02*
LDL (Normal: <130 mg/dL)			121.3±4.7			96.1±4.1	<0.001*
HDL Male (Normal: 40-50 mg/dL) Female (Normal: 50-60 mg/dL			42.7±2.5			50.1±3.5	<0.001
ESR (Normal: 0-15 mm/h)			83.2±5.5			64.5±4.1	0.01*
CRP (Normal <3 mg/L)			2.2±4.14			2.03±3.53	0.52
FBS (Normal: 65-104 mg/dL)			90±1.6			94±2.1	0.1
PCSK9 (Normal: 0.45-30 ng/mL)			146.7±6.8			115.3±7.4	0.003*
Interstitial lung disease	11	27		-	-		
Median MPAP (mmHg)	25			-			
Digital ulcers	9	22		-	-		
Modified Rodnan Skin score			23.1±1.3			-	-
European scleroderma study group			5.1±0.2			-	-

d-SSc: Diffuse systemic sclerosis; SD: Standard deviation; BMI: body mass index; TC: Total cholesterol; TG: Triglycerides; LDL: Low density lipoprotein; HDL: High density lipoprotein; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; FBS: Fasting blood sugar; PCSK9: Proprotein convertase subtilisin/kexin type 9; MPAP: Mean pulmonary arterial pressure.

Table 2. Correlation of study parameters with PCSK9

Parameters	r	*p*
mRSS	0.92	<0.001*
EScSG	0.87	<0.001*
Age	-0.2	0.2
Weight	0.04	0.79
Height	0.26	0.09
BMI	0.71	0.66
WC	0.5	<0.001*
SBP	0.243	0.12
DBP	0.081	0.61
Total cholesterol	0.39	0.011*
TG	0.37	0.01*
LDL cholesterol	0.65	<0.001*
HDL cholesterol	-0.35	0.02
ESR	0.23	<0.05
CRP	0.46	<0.001
FBS	0.096	0.55
MPAP	0.3	0.045
Mean CIMT	0.5	<0.001*

PCSK9: Proprotein convertase subtilisin/kexin 9; mRSS: Modified Rodnan skin score; EScSG: European scleroderma study group; BMI: Body mass index; WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglycerides; LDL: Low density lipoprotein; HDL: High density lipoprotein; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; FBS: Fasting blood sugar; MPAP: Mean pulmonary arterial pressure; CIMT: Carotid intima-media thickness.

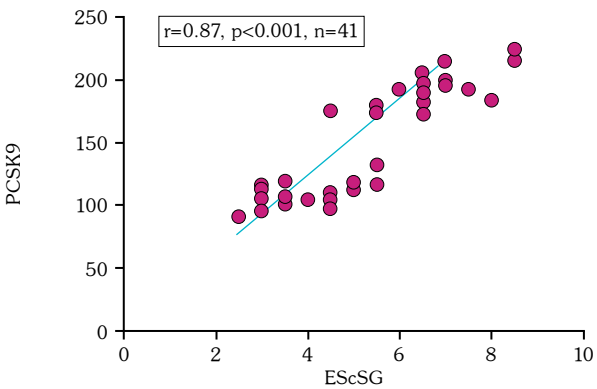

Figure 1. Correlation between PCSK9 and EScSG.

PCSK9: Proprotein convertase subtilisin/kexin 9; EScSG: European Scleroderma Study Group.

AUC for PCSK9 was 0.87 (95% CI: 0.73-0.96; p=0.0007; sensitivity= 90.1%; specificity= 73.5%) with a cut-off value of 172.8 ng/mL (Figure 3).

To identify factors associated independently with PCSK9, a multiple regression analysis was performed. The independent variables included WC, age, LDL-C, HDL-C, TG, systolic and diastolic blood pressures, FBG, ESR, CRP, EScSG score, mRSS, immunosuppressives including corticosteroids, azathioprine, mycophenolate mofetil, methotrexate, cyclophosphamide and hydroxychloroquine. The results showed that EScSG score (r=6.6, p=0.02), mRSS (r=2.4, p=0.001), HDL-C (r=0.4, p=0.01), LDL-C (r=0.47, p=0.02) and WC (r=0.84, p=0.002) were

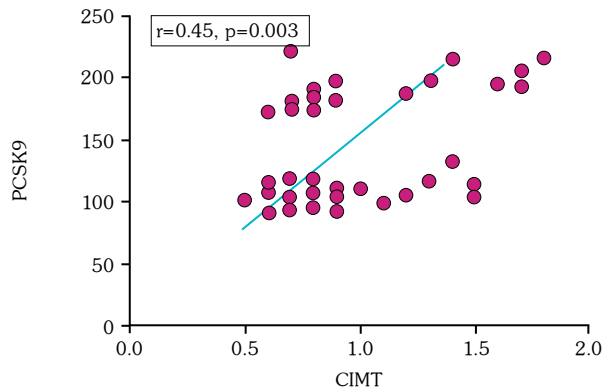

Figure 2. Pearson correlation between PCSK9 and mean CIMT.

PCSK9: Proprotein convertase subtilisin/kexin 9; CIMT: Carotid intima-media thickness.

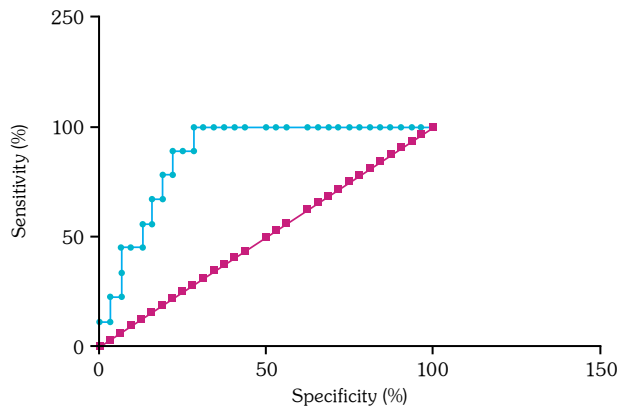

Figure 3. ROC curve for PCSK9 in digital ulcers in dSSc patients.

ROC: Receiver operating characteristic; PCSK9: Proprotein convertase subtilisin/kexin 9; dSSc: Diffuse systemic sclerosis.

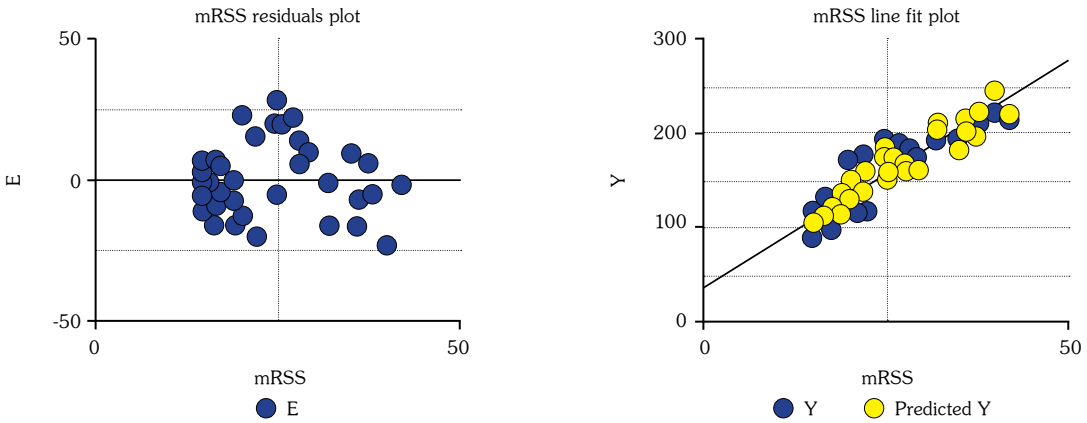

Figure 4. Modified Rodnan skin score association with PCSK9 in multivariate analysis.

mRSS: Modified Rodnan skin score; PCSK9: Proprotein convertase subtilisin/kexin 9.

significantly associated with PCSK9. Figure 4 shows the association of mRSS with PCSK9 in the multiple regression model.

DISCUSSION

Previous studies have shown that patients with d-SSCs have a significant risk for stroke, myocardial infarction, and CV death.[12] Additionally, CV mortality in d-SSc is accompanied by more severe manifestations of the disease and higher disease activity index.[13]

According to our results, serum TC, LDL-C, and TG levels were significantly higher while serum HDL-C was significantly lower in d-SSc patients compared to the control group. Several studies have reported consistent findings.[14,15] Impaired lipid profile in patients with scleroderma may be explained by the inflammatory process itself that may have an effect on HDL-C and LDL-C levels[14] and inhibition of lipoprotein lipase with subsequent high TG.[16] The PCSK9 inhibitors have been shown to effectively lower cholesterol levels by increasing the cellular uptake of LDL.[17] However; other researchers have found no significant difference in lipid profile between scleroderma patients and control group.[18,19] They explained their findings by the lack of differences in serum CRP levels and CV comorbidities between the study groups.

Compared to healthy controls, CIMT in our d-SSc patients was significantly increased. This is consistent with many previous studies reporting increased prevalence of subclinical atherosclerosis in d-SSc patients.[15,20,21] Endothelial dysfunction due to apoptosis, anti-endothelial antibodies, immune-mediated cytotoxicity, ischemia-reperfusion damage, and secretion of vasoconstrictor substances such as endothelin may stand behind the increased atherosclerotic risk in d-SSc.[22] On the other hand, others have shown no significant difference in CIMT between d-SSc patients and controls which can be explained by the lack of traditional CV risk factors in their d-SSc patients.[23-25] In the current study, there was a statistically significant increase in serum PCSK9 in d-SSc patients compared to controls. Many researchers have studied the possible link between PCSK9 and inflammation. Utilization of the cytoplasmic effects which can control the expression of genes that regulate inflammation in macrophages could be an explanation. Also, PCSK9 could aim LDL receptor-related protein-1 which is involved in Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and extracellular signal-regulated kinase (ERK) pathways activation. Finally, it functions as a key regulator of atherogenic inflammation by lowering the pathogenic lipid removal and connecting with the Toll-like receptor (TLR)/transcription factor nuclear factor-kappa B (NF-κB) pathway.[6,26-30]

In the field of SSc, to the best of our knowledge, Ferrazo-Amaro et al.[19] only discussed the relation

between PCSK9 and SSc. Unlike our findings, they found that PCSK9 was downregulated rather than upregulated in SSc patients. However, they included patients with both types of SSc: limited and diffuse types, which may misjudge the serum levels of PCSK9. Moreover, they reported that PCSK9 was elevated particularly in certain subgroup of d-SSc patients who had more severe systemic manifestations which is in line with our results of higher PCSK9 levels in those with DU, ILD and its correlation with PHT. Of note, the aforementioned study recommended further assessment in d-SSc which was investigated concisely by our own study. Taken together, we suggest that PCSK9 serum levels may be lower in limited compared to d-SSc for further studies.

The correlation between PCSK9 and lipid molecules in d-SSc patients is still controversial. In the current study, we found a significant positive correlation between PCSK9 and TC, LDL-C and TG, consistent with some previous studies.[30-33] This may be related to gain-of-function mutation of PCSK9 with subsequent increased levels of serum LDL-C,[34] increasing apolipoprotein synthesis through inhibition of the intracellular degradation of Apo-B[35] and/or degradation of very-LDL (VLDL) receptors by PCSK9.[36] On the other hand, other researchers have found no significant correlation between PCSK9 and lipid molecules[6,19] which can be explained by lipid paradox in inflammatory states. Furthermore, our results showed a significant positive correlation between PCSK9 and CIMT in d-SSc patients which is consistent with the study of Ferrazo-Amaro et al.[19] who concluded that PCSK9 could predict for a future CV risk and it could be a target for treatment of atherosclerosis in patients with scleroderma.

In the present study, serum PCSK9 levels positively correlated with disease activity as measured by EScSG scores, mRSS, and inflammatory markers including ESR and CRP. However, in the multivariate analysis, only EScSG scores and mRSS remained significantly associated with PCSK9 with adjustment for other factors, particularly lipid profile and other CV risk factors. More importantly, previous studies, consistent with our findings, showed a significant correlation of PCSK9 with disease activity in SSc,[19] RA,[5] SLE[6,37] and SpA.[7] Low levels of PCSK9 were also associated with remission in patients with RA treated with anti-tumor necrosis factor alpha (TNF-α).[38] Finally, in a case series, PCSK9 inhibitors were used successively in treatment of statin-associated immune-mediated necrotizing myositis.[39] Moreover, in the present study, we found a positive correlation between PCSK9 and some severe disease manifestations as high mRSS score, ILD, DU, and PHT consistent with Ferrazo-Amaro et al.'s[19] study. According to these findings, we studied the potential utility of PCSK9 as a diagnostic biomarker for prediction of DU and ILD; i.e., two severe disease manifestations, through the ROC curve analysis. In case of DU, the cut-off value of AUC for PCSK9 was 0.87 (95% CI: 0.77-0.97; p=0.0007; sensitivity= 77.7%; specificity= 81.2%) with a cut-off value of 182.6 ng/mL. For diagnosing ILD, the value of AUC for PCSK9 was 0.87 (95% CI: 0.73-0.96; p=0.0007; sensitivity= 90.1%; specificity= 73.5%) with a cut-off value of 172.8 ng/mL.

Limitations of the current study included its performance in a single tertiary center with a relatively small sample size. Based on our results as well as previous studies, we only suggest a possible benefit of PCSK9 inhibitors in d-SSc "at least in more severe disease manifestations" without using them in any of our patients. Hence, we recommend randomized controlled trials with larger sample size to study this hypothesis.

In conclusion, as in other autoimmune diseases with premature atherosclerosis such as RA, SLE and SpA, PCSK9 is a useful marker of disease activity in d-SSc and correlated well with severe disease manifestations and subclinical atherosclerosis. Besides its well-known anti-cholesterol effect, we suggest that PCSK9 inhibitors can be targeted in further larger, randomized-controlled trials as an optional treatment in these diseases at least in severe disease manifestations.

Ethics Committee Approval: The study protocol was approved by the Medical Research Committee Faculty of Medicine Cairo University Ethics Committee (date: 10.04.2022, no: ms-610-2021). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each participants.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, writing the article: M.A.H., Y.E.; Design: LR.; Control/supervision: M.A.H.; Data collection and/or processing: J.A.; Analysis and/or interpretation, references and fundings: Y.E., L.R.; Literature review: J.A., M.A.H.; Critical review: M.A.H.; Materials: J.A., L.R.

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